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(54) Title: *CORYNEBACTERIUM GLUTAMICUM* GENES ENCODING PROTEINS INVOLVED IN CARBON METABOLISM AND ENERGY PRODUCTION

(57) Abstract: Isolated nucleic acid molecules, designated SMP nucleic acid molecules, which encode novel SMP proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing SMP nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated SMP proteins, mutated SMP proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of SMP genes in this organism.

What is claimed:

1. An isolated nucleic acid molecule from *Corynebacterium glutamicum* encoding an SMP protein, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
2. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes an SMP protein involved in the production of a fine chemical.
3. An isolated *Corynebacterium glutamicum* nucleic acid molecule selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
4. An isolated nucleic acid molecule which encodes a polypeptide sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing,, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
5. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide selected from the group of amino acid sequences consisting of those sequences set forth in as even-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
6. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

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7. An isolated nucleic acid molecule comprising a fragment of at least 15 nucleotides of a nucleic acid comprising a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
8. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1-7 under stringent conditions.
9. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1-8 or a portion thereof and a nucleotide sequence encoding a heterologous polypeptide.
10. A vector comprising the nucleic acid molecule of any one of claims 1-9.
11. The vector of claim 10, which is an expression vector.
12. A host cell transfected with the expression vector of claim 11.
13. The host cell of claim 12, wherein said cell is a microorganism.
14. The host cell of claim 13, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
15. The host cell of claim 12, wherein the expression of said nucleic acid molecule results in the modulation in production of a fine chemical from said cell.
16. The host cell of claim 15, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.

17. A method of producing a polypeptide comprising culturing the host cell of claim 12 in an appropriate culture medium to, thereby, produce the polypeptide.
- 5 18. An isolated SMP polypeptide from *Corynebacterium glutamicum*, or a portion thereof.
19. The polypeptide of claim 18, wherein said polypeptide is involved in the production of a fine chemical.
- 10 20. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.
- 15 21. An isolated polypeptide comprising a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the amino acid sequence is not encoded
- 20 by any of the F-designated genes set forth in Table 1.
22. The isolated polypeptide of any of claims 18-21, further comprising heterologous amino acid sequences.
- 25 23. An isolated polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleic acid selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated nucleic acid molecules set forth in Table 1.
- 30 24. An isolated polypeptide comprising an amino acid sequence which is at least 50% homologous to an amino acid sequence selected from the group consisting of those

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sequences as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.

- 5 25. A method for producing a fine chemical, comprising culturing a cell containing a vector of claim 12 such that the fine chemical is produced.
26. The method of claim 25, wherein said method further comprises the step of recovering the fine chemical from said culture.
- 10 27. The method of claim 25, wherein said method further comprises the step of transfecting said cell with the vector of claim 11 to result in a cell containing said vector.
- 15 28. The method of claim 25, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
29. The method of claim 25, wherein said cell is selected from the group consisting of: *Corynebacterium glutamicum*, *Corynebacterium herculis*, *Corynebacterium lilium*,  
20 *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*,  
*Corynebacterium acetophilum*, *Corynebacterium ammoniagenes*, *Corynebacterium fujiokense*, *Corynebacterium nitrilophilus*, *Brevibacterium ammoniagenes*,  
*Brevibacterium butanicum*, *Brevibacterium divaricatum*, *Brevibacterium flavum*,  
*Brevibacterium healii*, *Brevibacterium ketoglutamicum*, *Brevibacterium*  
25 *ketosoreductum*, *Brevibacterium lactofermentum*, *Brevibacterium linens*,  
*Brevibacterium paraffinolyticum*, and those strains set forth in Table 3.
30. The method of claim 25, wherein expression of the nucleic acid molecule from said vector results in modulation of production of said fine chemical.
- 30 31. The method of claim 25, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine

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and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.

5 32. The method of claim 25, wherein said fine chemical is an amino acid.

33. The method of claim 32, wherein said amino acid is drawn from the group consisting of: lysine, glutamate, glutamine, alanine, aspartate, glycine, serine, threonine, methionine, cysteine, valine, leucine, isoleucine, arginine, proline, histidine,  
10 tyrosine, phenylalanine, and tryptophan.

34. A method for producing a fine chemical, comprising culturing a cell whose genomic DNA has been altered by the inclusion of a nucleic acid molecule of any one of claims 1-9.

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35. A method for diagnosing the presence or activity of *Corynebacterium diphtheriae* in a subject, comprising detecting the presence of one or more of SEQ ID NOs 1 through 782 of the Sequence Listing in the subject, provided that the sequences are not or are not encoded by any of the F-designated sequences set forth in Table 1,  
20 thereby diagnosing the presence or activity of *Corynebacterium diphtheriae* in the subject.

36. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the  
25 Sequence Listing, wherein the nucleic acid molecule is disrupted.

35. 37. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs in the Sequence Listing, wherein the nucleic acid molecule comprises one or more  
30 nucleic acid modifications from the sequence set forth as odd-numbered SEQ ID NOs of the Sequence Listing s.

38. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the regulatory region of the nucleic acid molecule is modified
- 5 relative to the wild-type regulatory region of the molecule.

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